



### Amendment to the Specification:

Please replace the paragraph at lines 2-6 on page 19 with the following paragraph:

The reporter gene also contains a binding site for  $\sigma 54$  RNAP. The consensus sequence for  $\sigma 54$  RNAP binding is 5'TGGCAC-N5-TTGCa/t 3' (SEQ ID NO.: 1). This sequence is located at -12 to -24 with respect to the start of transcription, whilst the more common sigma 70 recognition sequence is situated at -10 to/-35. Both the GG & GC must be on the same face of the DNA helix.

Please replace the paragraph under the heading "Strains" from lines 23-32 on page 23 to lines 1-4 on page 24 with the following paragraph:

TG1 $\Delta$ K was derived from TG1 (Gibson T. J. (1984), *Studies on the Epstein-Barr virus genome*, University of Cambridge) using the genome integration strategy of Haldimann A. et al., (1996) *Proc. Nat. Acad Sci USA* 93, 14361. Briefly, NifA (*K. pneumoniae*) residues 1-462 was amplified using Pfu polymerase (Stratagene) and primers 1 (5'- GAG TCA CTA ACG CAT ATG ATC CAT AAA TCC GAT TCG GAC -3') (SEQ ID NO.: 2), 2 (5' - CGC GGA TCC AAG CGG CCG CTC ATT AGC GAT GGT TGA ACA GAA TCA C -3') (SEQ ID NO.: 3) cut with NdeI and BamHI and cloned into the genome targeting suicide vector pSK50D-uidA2 (Haldimann. *Op. Cit.*) and transformed into the Pir<sup>+</sup> host strain BW23473 (Metcalf W.W. et al (1994) *Plasmid* 35, 1). Vectors were isolated and transformed into the Pir<sup>-</sup> host strain TG1 harbouring the plasmid pINT-ts (Hasan N. et al (1994) *Gene* 150, 51). Chromosomal integration was induced by a temperature shift to 42°C, which leads to expression of  $\lambda$  integrase from pINT-ts and simultaneously stops its replication. Integrants ~~where~~ were identified by Kanamycin resistance and screened for Nif coactivation. Once obtained TG1 $\Delta$ K was grown routinely without antibiotic selection.

Please replace the two paragraphs under the heading "Constructs" from lines 8-32 on page 24 to lines 2-14 on page 25 with the following two paragraphs:

Chimeric constructs were based on pDB737 (Austin S. et al (1994) *J Bacteriol.* 176, 3460, Buck M. et al (1986) *Nature* 320, 374) encoding NifA (*A. vinelandii*) under the control of the T7 promoter in the plasmid pT7-7 (Tabor S. & Richardson C.C. (1985) *Proc Natl Acad Sci USA* 82, 1074). Expression was by leakiness of the T7 promoter. Chimeras were constructed taking advantage of a[[ $\kappa$ ]] unique BanII cutting site, in the linker region between the central domain of NifA and the DBD. GCN4 was amplified

using Pfu polymerase (Stratagene) and primers 3 (5'- GCT GCC AGC GAG AGC CCG CCG CTC GCC GCG ATT GTG CCC GAA TCC AGT GAT CCT -3') (SEQ ID NO.: 4) and 4 (5'- GAG CTA AAG CTT TTA TTA GCG TTC GCC AAC TAA TTT CTT TAA TCT GGC -3') (SEQ ID NO.: 5) cut with BanII and Hind3 and ligated into pDB737 cut with BanII and Hind3. ERDBD was amplified using primers 5 (5'- GTC GAC AAC GAG AGC CCG CCG CTC GCC GCG GAA ACG CGT TAC TGC GCT GTT -3') (SEQ ID NO.: 6) TGC and 6 (5'- GGT CAG CGC GTG GAT CCT TAA CCA CCA CGA CGG TCT TTA CG-3') (SEQ ID NO.: 7), cut with BanII and BamHI and ligated into pDB737 cut with BanII and BamHI. The vector p737S1 is derived from pDB737 by replacing the bla gene with aadA conferring streptomycin resistance and the insertion of a f1 phage origin for packaging of the vector into filamentous phage particles. Briefly, aadA was amplified using primers 7 (5'- TCA GCG CAC GCT GAC GTC GTG GAA ACG GAT GAA GGC ACG AAC -3') (SEQ ID NO.: 8), 8 (5'- CCG CCT GGA GGT GGC CAT TAT TTG CCG ACT ACC TTG GTG ATC TCG CC -3') (SEQ ID NO.: 9) and cut with AatII and MscI and ligated with pDB737 cut with AatII and ScaI. The resulting vector p737S was cut with AatII, ClaII. The f1 ori was amplified using primers 9 (5'- GCT GCC GAC TCG ATC GAT GAA TGG CGA ATG GCG CCT GAT GCG G -3') (SEQ ID NO.: 10), 10 (5'- CCG GGT CGT GAC GTC AGT GTT GGC GGG TGT CGG GGC TGG C -3') (SEQ ID NO.: 11) cut with AatII, ClaI and cloned into the cut p737S to give p737S1. NifA-X chimera were transferred from pDB737 to p737S1 by digestion with NdeI, Hind3 (BamHI for NifA-ERDBD).

Reporter constructs were derived from pACYC184 and the vector pMB1 (Buck M. *et al.* (1986) *Nature* 320, 374). Briefly the lac-operon (lacZYA) was amplified with primers 11 (5'- GAG TCA ATT CGG GGA TCC CGT CGT TTT ACA ACG TCG TGA CTG G -3') (SEQ ID NO.: 12), 12 (5'- GAG TCA TTC TGG CCA GTC GAC CGC TCT GCC GGT GGT TAC -3') (SEQ ID NO.: 13) and cut with BamHI and MscI. The nifH promoter segment from pMB1 was amplified with primers 13 (5'- GAG TCA TTC AAG CTT GCG TGG AAT AAG ACA CAG GGG GCG -3') (SEQ ID NO.: 14), 14 (5'- GAG TCA TTC GGG ATC CCC GGA TTT ACC GAT ACC GCC TTT ACC -3') (SEQ ID NO.: 15) and cut with Hind3, BamHI and the 2 fragments simultaneously ligated with pACYC184 cut with Hind3 and BsaAI to give pMB3. The f1 ori was amplified with primers 15 (5'- GCT GCC GAC TCG GCT AGC GAA TGG CGA ATG GCG CCT GAT GCG G -3') (SEQ ID NO.: 16), 16 (5'- GCC GGG TCG CTT TAA AGT GTT GGC GGG TGT CGG GGC TGG C -3') (SEQ ID NO.: 17) and cut with NheI and DraI and ligated into pMB3 cut with both NheI, XmnI to give pMB31.

Applicants respectfully request the attached paper copy of the Sequence Listing be inserted into the application.